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DSC serum profiles of sportsmen

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Abstract The blood serum of young athletes has been investigated by differential scanning calorimetry (DSC) method. The component peaks of complex endothermic transition corresponding to serum denaturation may be correlated with the main protein fractions of serum: albumin, alpha-1, alpha-2, beta- and gamma-globulins. The averaged shape of thermal transition observed for serum of sportsmen participating in our pilot study differs from that reported previously for healthy subjects. One of the reason for the difference may be the lower than normal value of albumins to globulins ratio found for individuals in our group of athletes. Modifications of serum DSC transition due to the exercise test and 1 and 2 h rest have been observed. Statistically based conclusions are not currently possible because of the low number of participants in the study. The averaged responses to the effort and to the rest have been shown clearly different for athletes classified as the best and typical. The first moment of the transition with respect to the temperature axis has been found significantly lower for the group of athletes considered as the best.

Keywords Differential scanning calorimetry (DSC) · Blood serum · Protein denaturation · Sport medicine

Introduction

The ability of sport scientists to predict which athletes amongst an elite group will become world-class is limited. We do not fully understand the interactions among biological factors, training, recovery and competitive performance. To date, the physiological dangers of sporting overexertion, the overtraining phenomenon in endurance sports are still the challenging research subjects in sports medicine. The occurrence of overtraining is not easily recognized and the differentiation between well-trained and overtrained athletes is difficult. A multidisciplinary and interdisciplinary approach is necessary to resolve mentioned above and other important problems of contemporary sport medicine.

Recent studies have shown that endurance overtraining could result from successive and cumulative alterations in metabolism [1]. While no single marker can be taken as an indicator of impending overtraining, the regular monitoring of a combination of performance, physiological, biochemical, immunological and psychological variables would seem to be the best strategy. Efforts are also being directed towards the development of new custom methods based on relatively easily accessible biological samples. Although plasma and serum from human blood are widely used in medical diagnosis, their diagnostic capability is not yet fully exploited. FT IR spectrometry has been used as a new analytical method to analyze plasma contents for the longitudinal biological monitoring of athletes [1, 2] mainly for identifying athletes at risk of developing overtraining syndrome. For global analysis of plasma or serum the differential scanning calorimetry (DSC) can also be applied. This technique was shown recently as valuable tool for disease diagnosis [3–9].

In this study, the DSC has been used for recognizing the thermal profiles of sportsmen serum. The long-term aim of our preliminary study is the detection of the *onset* of

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overtraining by global analysis of serum DSC profile. DSC method has not been probably applied for diagnosis of overtraining yet. The current aim was the comparison of serum DSC curves and thermodynamic parameters describing serum denaturation transition for samples taken from young athletes before and after the exercise test and after the rest lasting 1 and 2 h.

Experimental

Participants of the experiment

The research material included 11 male amateur cyclists; age range 17–25 years, body height 179 (SD 5) cm, body mass 74 (SD 6) kg. All subjects were tested in the laboratory before and after the progressive test. The progressive test was performed on an ergocycle Excalibur Sport (Lode BV, the Netherlands), beginning with a workload of 40 W, which was increased by 40 W every 3 min until volitional exhaustion. During the test, the following variables were constantly registered: heart rate (HR) minute ventilation (VE), oxygen uptake (VO_2) and expired carbon dioxide (CO_2) (MetaLyzer 3B-R2, Cortex, Germany). The maximal oxygen consumption ($\text{VO}_{2\text{max}}$) was determined to extrapolate the performance level. The criteria of reaching $\text{VO}_{2\text{max}}$ included a plateau in values of VO_2 or a gradual decrease in peak VO_2 during the maximal workload. The mean relative value of $\text{VO}_{2\text{max}}$ was 52 (SD 8) $\text{mL kg}^{-1} \text{min}^{-1}$. The mean maximal workload (WR_{max}) was 308 (SD 63) W. Four cyclists were classified as the best taking into account the performance level— $\text{VO}_{2\text{max}} \geq 62 \text{ mL kg}^{-1} \text{min}^{-1}$, $\text{WR}_{\text{max}} \geq 320 \text{ W}$, and the cycling experience as less important criterion.

The atmospheric conditions in regards to air pressure, temperature and humidity were held constant to increase the reliability of measurements.

Blood serum samples

Blood samples were taken prior to and after the exercise and then after 1 h and 2 h of the rest. Blood samples were analyzed for albumin, α_1 -, α_2 -, β - and γ - globulins and for total protein concentration. Haematocrit (%) was measured and used to estimate changes in plasma volume.

Serum samples were stored at -20°C before analysis. For DSC experiment serum samples were diluted 1:20 with distilled water. The pH value of the diluted samples has been within the range 6.5–7.5.

DSC experiment

DSC measurements were carried out on the VP DSC MicroCal instrument (Northampton, MA) with heating rate

of 1°C min^{-1} and with a pre-scan equilibration time of 15 min. Heat capacity, C_p , versus temperature profiles were obtained in the temperature range 20–100 $^\circ\text{C}$. The water–water scan was used as an instrumental baseline. Serum DSC curves were normalized for the gram mass of protein and next linear baseline was subtracted. The following thermodynamic parameters have been found: the temperatures of local peak maxima (T_1 , T_2 , T_3), the enthalpy (ΔH) of serum denaturation (calculated as the area under the peak, expressed in J g^{-1}), the width of peak in its half height (HHW) and the normalized first moment (M_1) of the thermal transition with respect to the temperature axis calculated as:

$$M_1 = \frac{\int_{T_i}^{T_f} TC_p dT}{\int_{T_i}^{T_f} C_p dT}, \quad (1)$$

where T_i and T_f are the beginning and end T s of denaturation, respectively.

In addition, C_{p3}/C_{p1} , C_{p3}/C_{p2} —ratios of excess specific heat capacities C_p at temperatures T_3 , T_1 and T_3 , T_2 , respectively—have been calculated.

Statistical analysis

Statistical analysis of results was done with Statistica 9 using ANOVA or non-parametric Kruskal–Wallis test. The limit for statistical significance was set at 0.05.

Results and discussion

The denaturation of human blood serum proceeds over the temperature range 40–90 $^\circ\text{C}$. The complex endothermic transition observed for serum arises from the denaturation of the constituent proteins and represents the weighted sum of denaturation profiles of the individual proteins within serum. The transition is irreversible. The changes of protein concentration levels, disturbances of protein structure as well as intermolecular interactions amongst different proteins and other components are reflected in the shape of denaturation peak and in thermodynamic parameters describing serum transition.

In Fig. 1, the mean DSC curve (the excess specific heat capacity as a function of temperature) of serum coming from sportsmen before the exercise test is shown. It may be compared with the mean plasma or serum DSC curves reported previously for healthy individuals [3–7]. Generally, plasma and serum curves should be very similar, although the kind of solvent used for serum dissolution may be an important origin of differences. Differences

between plasma and serum are mainly due to fibrinogen content in plasma and its lack in serum (it is removed when plasma is allowed to clot). Two main peaks have been revealed in fibrinogen DSC profile: at about 51 and 93 °C [3].

For healthy individuals the maximum of serum or plasma transition at about 62 °C and a shoulder in the higher temperature range, at about 70 °C, have appeared [3–7]. Whereas for averaged serum transition observed for men participating in our study, three local maxima are visible (Fig. 1). The peak with maximum at about 70 °C dominates while these at about 57 and 62 °C are less intensive. The mean thermodynamic parameters for denaturation transition of athlete's serum are listed in Table 1.

Comparing the average DSC serum curve obtained in this study with the earlier results for healthy individuals one can ask whether athletes are healthy. Such question arises because the shape of the transition and the value of its first moment M_1 are more similar to these observed in disease states. Generally, the thermal transitions observed for serum/plasma of individuals suffering from various diseases have been found significantly shifted towards higher denaturation temperatures when compared with healthy controls. Another characteristic feature of these transitions, indicating disease state, is a decrease in the amplitude of the major peak at about 62 °C with concomitant increase in amplitude of the peak at about 70 °C and its higher temperature shoulders. An increase of intensity of the peak at 70 or 80 °C is characteristic for lung cancer, breast cancer, melanoma, chronic obstructive pulmonary disease (COPD) and other diseases [3–6].

The athletes are usually thought to be physically normal and health by definition, but high workload for training and psychophysical stress due to competitions could modify the homeostasis, inducing apparently pathological biochemical

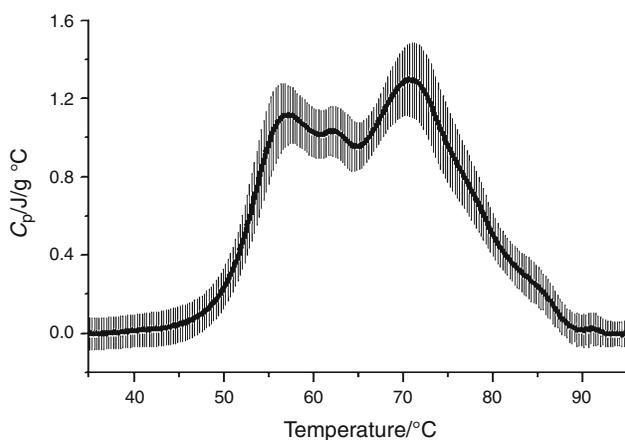


Fig. 1 Mean DSC curve ($n = 11$) of sportsmen serum before the exercise test. The shaded area is the standard deviation at each temperature

Table 1 The thermodynamic parameters of denaturation transition for serum of athletes acquired before and after exercise and after 1 and 2 h of rest

	Before exercise	After exercise	Rest 1 h	Rest 2 h
$T_1/^\circ\text{C}$	57.2 ± 0.3	57.3 ± 0.3	56.6 ± 0.3	56.8 ± 0.5
$T_2/^\circ\text{C}$	62.3 ± 0.2	62.4 ± 0.2	62.1 ± 0.2	62.2 ± 0.2
$T_3/^\circ\text{C}$	70.7 ± 0.1	70.7 ± 0.1	70.7 ± 0.1	70.8 ± 0.1
$HHW/^\circ\text{C}$	24.9 ± 0.4	25.1 ± 0.4	25.6 ± 0.4	25.5 ± 0.5
$\Delta H/J \text{ g}^{-1}$	31.4 ± 0.4	33.1 ± 0.8	31.8 ± 0.8	30.6 ± 0.8
$M_1/^\circ\text{C}$	66.4 ± 0.2	66.3 ± 0.2	66.0 ± 0.3	66.3 ± 0.3
C_{p3}/C_{p1}	1.16 ± 0.06	1.05 ± 0.05	1.09 ± 0.07	1.19 ± 0.06
C_{p3}/C_{p2}	1.25 ± 0.05	1.18 ± 0.04	1.21 ± 0.05	1.30 ± 0.04

and haematological values. The strenuous endurance training may result in a fatigue accumulation. One consequence is a greater susceptibility to various pathologies, such as asthenia, upper respiratory tract infections (and viral or bacterial infections).

Although the levels of proteins in serum determined in the group of men participating in our study have fallen within the normal range, we have noticed that the albumins to globulins (A/G) ratio was very low: 1.1 ± 0.1 while the correct value is from the range 1.7–2.2. The A/G ratio may be decreased for instance in liver dysfunction.

It appears that the correlation between intensities of component peaks and protein fractions can be found (see Table 2). The first peak is correlated with albumin and alpha-2 globulins, the second is correlated with alpha-1- and alpha-2-globulins and third is correlated with beta- and gamma-globulins. In addition, C_{p1}/C_{p3} ratio is highly correlated with A/G ratio, Pearson's coefficient above 0.7 (statistically significant, $p < 0.01$). Thus, an increase or decrease of component peaks intensity may be linked to changes in various protein fractions.

Figure 2 illustrates the differences between averaged DSC curves of serum taken before exercise, after exercise, after an hour of rest and 2 h of rest. The intensity of serum denaturation peak increases due to the effort and diminishes after the rest. The curves corresponding to pre-exercise serum and after 1 h of rest are very similar. Amongst mean thermodynamic parameters listed in Table 1, T_1 tends to be lower after the rest, ΔH shows the tendency to be higher after the exercise, C_{p3}/C_{p1} , C_{p3}/C_{p2} ratios show the tendency to decrease after the exercise and to rise after the rest. However, no differences are statistically significant. These results indicate that the mean changes connected with an exercise or rest are not statistically important. The large experimental group is needed to confirm the effect of an effort and rest on DSC profile of athlete's serum. The presence of such effect is suggested by a comparison of the averaged DSC curves shown in Fig. 2. An increase of

Table 2 Pearson's correlation coefficients between intensities of the local peak maxima and the contents of protein fractions in athlete's serum

	Albumin	Alpha-1 globulins	Alpha-2 globulins	Beta globulins	Gamma globulins
C_{p1}	0.46 $p = 0.01$	0.25 $p = 0.19$	0.41 $p = 0.03$	-0.18 $p = 0.35$	-0.09 $p = 0.63$
C_{p2}	0.23 $p = 0.24$	0.39 $p = 0.04$	0.40 $p = 0.03$	0.02 $p = 0.93$	0.18 $p = 0.36$
C_{p3}	-0.11 $p = 0.58$	0.34 $p = 0.08$	0.03 $p = 0.87$	0.68 $p = 0.00$	0.63 $p = 0.00$

Significant correlations ($p < 0.05$) are printed in bold

the intensity of peaks at 57 and 62 °C after the exercise indicates an increase of albumin and α_1 -, α_2 -globulins level in athlete's serum. In fact, the mean albumin concentration in serum of athletes participated in this study has increased from $37.5 \pm 1.3 \text{ g L}^{-1}$ before the exercise test to $40.9 \pm 0.6 \text{ g L}^{-1}$ (or $39.5 \pm 1.0 \text{ g L}^{-1}$ taking into account changes in plasma volume) after the test. The changes in albumin concentration have been found statistically significant. The elevated level of albumin in serum after exercise is probably connected with dehydration due to very intense perspiration during the exercise. There are no significant differences between determined mean levels of globulins in pre- and post-exercise serum as well as after the 1 h and 2 h rest. However, for all types of globulins the tendency to increase after an effort has been observed.

Comparing Fig. 3a, b, one can see clearly differences between the best and typical athletes in the averaged responses to the effort and to the rest. The biggest difference has appeared in the shape of DSC curves after 2 h of rest. Notably, in this case, the peak at about 57 °C correlated with the content of albumin and α_2 -globulins (Table 2) is greatly diminished for typical athletes but not for the best. Albumin level after 2 h of rest has been determined as normal and very similar in both groups

(about 39 g L^{-1}). The level of α_2 -globulins has been measured actually lower after the rest for typical athletes.

Statistical analysis of thermodynamic parameters has indicated significantly lower values of T_1 , T_3 , and M_1 (see also Fig. 4 for the comparison of M_1) for the group of the best athletes. No important differences between both groups of athletes have been found for the enthalpy of serum denaturation.

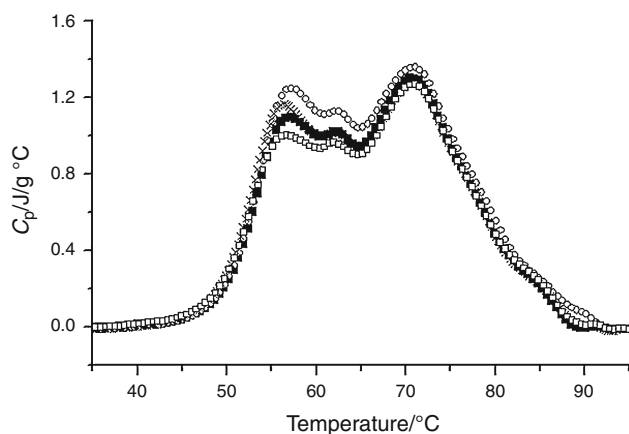


Fig. 2 Mean DSC curves of serum acquired from 11 athletes before (solid squares) and after (open circles) exercise test and after 1 h (crosses) and 2 h (open squares) of rest

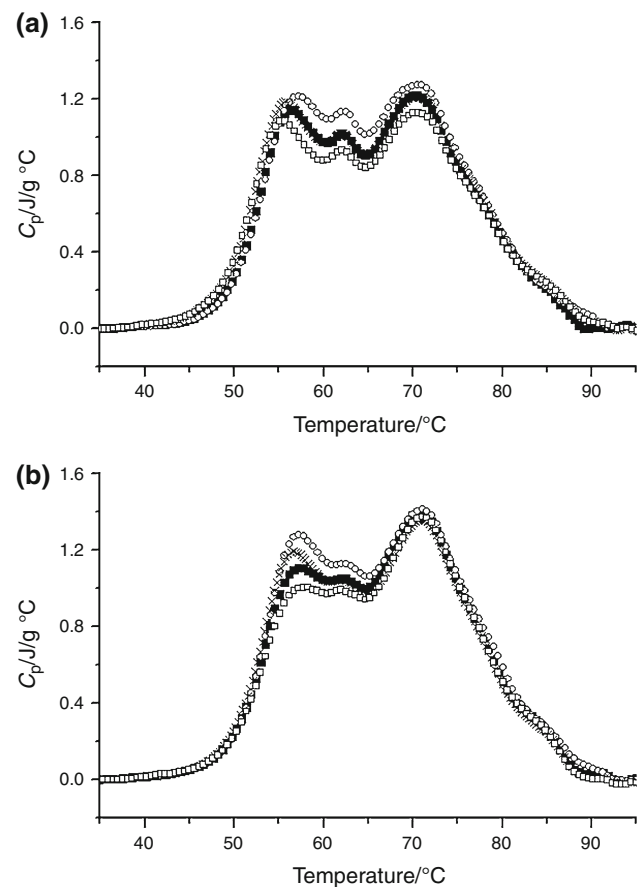


Fig. 3 Mean DSC curves of serum acquired from 4 the best athletes (a) and 7 typical athletes (b) before (solid squares) and after (open circles) exercise test and after 1 h (crosses) and 2 h (open squares) of rest

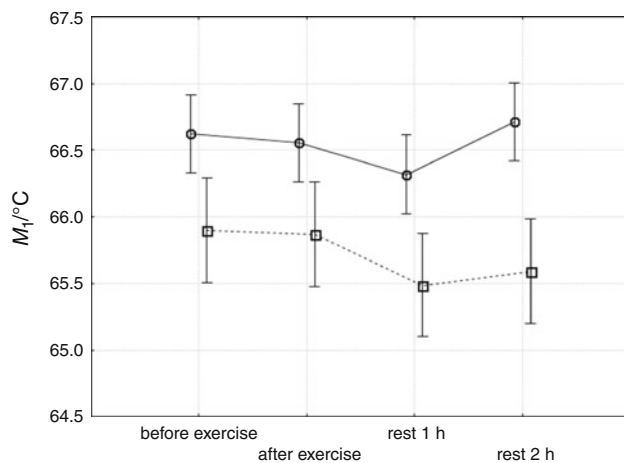


Fig. 4 The first moment (M_1) of the transition with respect to the temperature axis for serum of the best (squares) and typical (circles) athletes

Probably, neither an albumin nor gamma-globulin levels are the origin of differences between two groups of athletes. Statistically important differences between the best and typical athletes have been found only in the levels of alpha-2- and beta-globulins. The level of alpha-2 globulins has been observed higher while the level of beta globulins lower for the best than for the typical group (data not shown). It is worth mentioning that for typical athletes the levels of alpha-1- and beta-globulin have increased slightly above the optimal range (to 3.5 ± 0.1 and to $12.6 \pm 1.0 \text{ g L}^{-1}$, respectively) after the exercise test.

Conclusions

Alterations and interactions within the plasma/serum proteome give rise to changes in DSC plasma/serum thermal profile. Global analysis of plasma or serum DSC curve in the temperature range of protein components thermal denaturation can support diagnosis of human diseases [3–9]. Similar analysis of sportsmen serum would probably facilitate the resolving of some problems in sport medicine.

The shape of DSC curves observed for serum of young athletes participating in this pilot study differs from that characteristic for healthy subjects. The peak with maximum at about 70 °C dominates in thermal transition of sportsmen serum, while for healthy serum the dominant peak has been found that with maximum at about 62 °C. Near the temperature 70 °C only the shoulder has been observed. An increase of that shoulder intensity has been reported in some disease states [3–6]. For individuals in our group of athletes the albumins to globulins ratio was lower than normal value although the levels of proteins in serum have fallen within the normal range.

Significant correlation have been shown between intensities of local maxima in complex serum transition peak and protein fractions levels within serum. The first component peak has been correlated with albumin and alpha-2 globulins (e.g. haptoglobin), the second has been correlated with alpha-1- and alpha-2-globulins and third has been correlated with beta and gamma-globulins. Thus, an increase or decrease of component peaks intensity can provide relationships with changes in various protein fractions.

Distinct changes in serum DSC curves have been observed due to the exercise and rest, but small experimental group and large variability between individuals make impossible to show statistical significance of these changes for particular stages of the experiment. There are, however, noticeable differences between denaturation transitions observed for serum of athletes classified as the best and typical. Actually, statistically important differences in the levels of alpha-2- and beta-globulins have been found between athletes assigned to these groups. The averaged responses to the exercise and to the rest have been shown clearly different for both groups of athletes. The biggest differences have been revealed after 2 h of rest. The first moment (M_1) of the transition with respect to the temperature axis, the most important parameter, which allows to distinguish health and disease based on DSC results, has been found significantly lower for athletes considered as the best. Lower M_1 values suggest better fitness and health of individuals.

We hope that our initial results of athlete's serum DSC study indicate that this technique may be applied specifically to sport medicine studies. Further experiments with increased number of participants are planned.

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